PAPP-A as a marker of increased long-term risk in patients with chest pain

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Abstract

Objectives—Long-term risk stratification in patients presenting with acute coronary syndromes (ACS) is possible by measuring cardiac troponin (cTn). The present study examined whether PAPP-A measured in an emergency department (ED) chest pain population in association with conventional and novel high sensitivity cTn (hs-cTnI) assays can predict long-term mortality.

Methods—In 320 patients with cTn measurements the earliest heparinized plasma PAPP-A concentration after presentation was used for risk stratification for death by Kaplan–Meier and Cox analyses. Subgroup analyses using the earliest PAPP-A concentrations were also performed in a cohort of subjects with presentation cTnI 99th percentile but with significantly changing cardiac troponin concentrations as measured by the AccuTnI assay and the hs-cTnI assay ($n=45$ and 120 subjects, respectively).

Results—Subjects with PAPP-A concentrations in the highest tertile were at higher risk for death (HR>2.00; $p < 0.05$ at 2 years) even after adjusting for cTnI at presentation. In the cohort with cTnI 99th percentile but with changing hs-cTnI concentrations, subjects in the top PAPP-A tertile had a higher probability for death ($p=0.02$).

Conclusion—Early measurement of PAPP-A may identify chest pain patients at higher risk for long-term death. Additional prospective ACS studies are required to fully elucidate PAPP-A's role.

Keywords

Pregnancy associated plasma protein A; Cardiac troponin; Death; Emergency department; High sensitivity

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Introduction

Acute coronary syndromes (ACS) including unstable angina, non-ST and ST-elevation myocardial infarction are the leading causes of mortality and morbidity worldwide [1,2]. Despite sophisticated imaging technology and biochemical markers to assist in the diagnosis and monitoring of ACS patients, there still are patients who are inaccurately diagnosed in the emergency department (ED) and others whose risk of adverse events following presentation is underestimated [3–7]. The use of sensitive troponin assays has helped with better diagnosis [8], but additional biomarkers would be especially useful to identify patients at long-term risk after presentation. A few acute-care markers such as cardiac troponin (cTn), natriuretic peptides and C-reactive protein (CRP), have been shown to have longer-term predictive value in patients with ACS [7,9–16] but there are additional patients at risk who are not identified, perhaps in part because none of these biomarkers provides information about atherosclerotic plaque instability. A sensitive and specific early biomarker of atherosclerotic plaque instability would assist in risk stratification. Vulnerable coronary atherosclerotic plaques have been reported to contain pregnancy associated plasma protein-A (PAPP-A) [17,18]. Since these lesions appear to precede the development of myocardial ischemia and necrosis, PAPP-A could serve as a useful marker both for early diagnosis of ACS and to define those without acute events who are at subsequent risk for events [18,19]. In the present study, we used a new high-sensitivity PAPP-A (hsPAPP-A) assay to assess both the analytical and clinical utility of PAPP-A in an emergency department chest pain population characterized with a conventional sensitive cTnI assay and new research high-sensitivity cTnI (hs-cTnI) assay.

Methods and materials

Study population and laboratory analyses

The study population has been previously described [14,15,20–22]. Briefly, in 1996 after research ethics approval, 458 patients representing 500 separate patient presentations to the ED were enrolled in a Cardiac Markers study. The only inclusion criterion was the assessment by the triage staff that the patient had symptoms suggestive of cardiac ischemia; there were no exclusion criteria. Heparin plasma samples were collected at presentation and at scheduled intervals measured from the time of pain onset: hourly until 6 h after onset, then at 9, 12, 24, and 48 h or until the patient was discharged, declined further participation, or was removed from the study by those responsible for his/her care. Specimens were stored until 2003 when cTnI (AccuTnI™ Beckman Coulter) was measured in all available specimens.

In 2007 all remaining heparin plasma specimens (stored at –80 °C) with sufficient volume were thawed a second time and measured first by a highly sensitive research cardiac troponin I assay (hs-cTnI with a limit of detection of 2.06 ng/L, Beckman Coulter) [22] then with a hsPAPP-A ELISA (DSL-90-27600; Beckman Coulter) that measures uncomplexed PAPP-A and the PAPP-A/proMBP complex in equimolar concentration, but not proMBP nor other cross reactants such as PAPP-A2, MMP-9 and MMP-12 (both serum and heparin plasma are suitable sample types for this assay). The manufacturer has determined the lowest detectable level of PAPP-A (mean signal of 16 replicates of the zero calibrator plus

two standard deviations) with 95% confidence to be 0.184 mIU/L. However, for this analysis we did not confirm the lowest detectable level, rather for any concentrations below the lowest calibrator (0.50 mIU/L), 0.49 mIU/L was assigned to these values for statistical purposes ($n=51$ or 5% of 1081 results). There were 1081 heparin plasma specimens from 357 patient presentations (320 unique subjects) that were measured with the PAPP-A assay; 70% of these specimens had sufficient volume for duplicate PAPP-A measurements in which case the average concentration was used. In cell culture systems, animal models and patients with STEMI it has been observed that treatment with heparin elevates circulating PAPP-A concentrations [23,24], and in our population 70 of the 320 subjects (22%) received heparin at some time during their hospital stay. To mitigate the potentially confounding effects of heparin on PAPP-A levels we used the earliest available specimen for outcome analysis. One third of the subjects had a PAPP-A measured after the 1st cardiac troponin ($n=107$; median (IQR) time after 1st specimen=2 (1–6) hours), however only 19 of these subjects received heparin. The total imprecision (CV; coefficient of variation, $n=38$) for the hsPAPP-A assay during the study at two quality control levels provided with the assay (4.82 and 12.67 mIU/L) was 14.2% and 10.6%, respectively; however, for lower concentrations, the manufacturer states a total imprecision of 9.9% at a PAPP-A concentration of 1.73 mIU/L. This is consistent with the duplicate CVs from our population (median concentration (IQR)=1.66 (1.03–3.26); median CV (IQR)=7% (3–14).

A cohort of subjects (186 from 320) was identified who had both AccuTnI and hs-cTnI measurements performed on multiple specimens ($n=570$ specimens with both cTnI measurements), and for whom the presentation AccuTnI was 99th percentile (0.04 $\mu\text{g/L}$), and for whom a concentration change in cTnI was observed, either for AccuTnI ($n=45$) or for hs-cTnI ($n=120$), respectively (see Fig. 1). Briefly, an analytically significant concentration change was noted if the difference between the highest and the lowest cTn concentrations observed within a subject was >3 SD or $>20\%$, consistent with the 2007 MI definition and our previous work [2,22]. The earliest PAPP-A concentration was used for risk stratification in this cohort as well.

Health outcomes and statistical analysis

After research ethics board approval, mortality outcomes were obtained via linkage to the Registered Persons Data Base (RPDB) [14,15]. The outcomes were captured as events post-presentation (i.e., either during the index hospitalization or afterwards). Between-group comparisons of central tendency (means, medians) were based on one-way analysis of variance (ANOVA), Mann Whitney and the Kruskal–Wallis tests. The nonparametric Spearman correlation coefficient was determined for PAPP-A, cTnI (AccuTnI) and hs-cTnI. The Pearson Chi-square test statistic was used to compare proportions.

The time to death was assessed by tertile analysis of the earliest PAPP-A value by Kaplan–Meier survival curves for up to 2 years and 10 years, with differences between groups determined by the log rank test. In keeping with our previous analyses the covariates and cutoffs chosen for the Cox proportional hazard models were the same [14,15]. Specifically, tertiles 2,3 were compared to tertile 1 (referent) in various models: model 1 adjusted for age and sex; model 2 adjusted for age, sex, and presentation AccuTnI 0.02 $\mu\text{g/L}$ (i.e., detectable

cardiac troponin) with the hazard ratios (HR) derived by partial likelihood estimation with the significance of the association based on the Wald Chi-square statistic. All statistical analyses were performed using SAS and Graphpad Prism with a p -value 0.05 considered statistically significant.

Results

The median age was 64 years for the study population ($n=320$ with 60% men; see Table 1). The values for PAPP-A appear to manifest a rise during the first 9 h after pain onset but there is a considerable overlap in PAPP-A ranges between the different time points (Fig. 2A). There was no difference in the initial PAPP-A concentrations in those who received ($n=70$) and did not receive heparin ($n=250$) during their hospital stay (median (IQR) PAPP-A=1.22 (0.80–1.99) vs. 1.20 (0.79–1.97) mIU/L; $p=0.68$), however the peak PAPP-A was significantly higher in those who received heparin vs. no heparin (median (IQR) PAPP-A=4.03 (1.95–9.84) vs. 1.56 (0.95–2.96) mIU/L; $p<0.01$). In those who did not receive heparin, there was a significant yet weak correlation between the earliest PAPP-A and cTn ($r<0.15$; $p<0.05$) (Table 2). Assessing the baseline characteristics across the PAPP-A tertiles only sex and hs-cTnI were different among the groups (see Table 3 and Fig. 2B for PAPP-A concentrations).

Kaplan–Meier analysis by tertiles with the earliest available PAPP-A measurement (i.e., at baseline) demonstrated that the higher PAPP-A concentrations were associated with a higher probability of death within 10 years (Figs. 3A,B). At 2 years, Cox proportional hazard analyses based on the tertiles, indicated that those subjects with PAPP-A concentrations in the upper third were at significantly higher risk for death, even after adjusting for age, sex, and baseline cTnI, all previously shown to enhance the predictive accuracy of these markers (Table 4).

In the 186 subject cohort without elevated presentation AccuTnI (i.e., cTnI 99th), for whom all available specimens were re-analyzed with the high sensitivity research hs-cTnI assay, 120 individuals had an analytically significant concentration change by the hs-cTnI assay and 45 had a change with the AccuTnI assay. Kaplan–Meier analysis of tertile PAPP-A baseline concentrations for two-year survival was not significant in subjects with the changing pattern manifested by the AccuTnI assay ($p=0.47$); however, PAPP-A was a significant marker for survival in the subjects with changes detectable by the hs-cTnI assay ($p=0.02$) (Fig. 4). However, PAPP-A was not useful in patients without a changing hs-cTnI pattern ($p=0.90$). Furthermore, after removing subjects who received heparin from the 186 cohort ($n=46$), those who had a changing hs-cTnI concentration ($n=89$) had a higher PAPP-A concentration as compared to those who had no change ($n=51$) (peak PAPP-A=1.88 mIU/L (1.06–3.66) vs. 1.29 (0.86–2.27); $p=0.03$).

Discussion

PAPP-A is a 200 kDa metalloproteinase that circulates in distinct forms during pregnancy and ACS [25]. During pregnancy, PAPP-A circulates as a heterodimer with the pro-form of eosinophil major basic protein (proMBP). Bound proMBP acts as a protease inhibitor

[19,26]. PAPP-A is found in unstable plaques, however the physiological role of PAPP-A in the atherosclerotic plaque is not clear. One hypothesis is that PAPP-A secretion is a repair mechanism involved in plaque stabilization. PAPP-A is secreted by vascular smooth muscle cells and cleaves insulin-like growth factor binding protein-4 (IGFBP-4) in an IGF dependent manner to amplify local bioactive IGFs. IGFs stimulate cell proliferation and differentiation resulting in tissue salvage and repair [27,28]. Conversely, it has been proposed that the metalloproteinase action of PAPP-A causes degradation of the extracellular matrix and may also mediate inflammatory events in atherogenesis thereby contributing to plaque destabilization [29,30]. Further examination of the role of PAPP-A in plaque rupture is required to elucidate its function. Regardless of the cause, previous studies have indicated that PAPP-A is a strong independent predictor of short-term (i.e., 6 months) cardiovascular events in patients with acute chest pain or ACS, even in those without cardiac troponin elevations [31,32]. However, those studies used higher cardiac troponin cutoffs (WHO cutoffs or 10% CV concentration cutoffs) than recommended by contemporary guidelines.

The present study extends previous reports by investigating an ACS population characterized with a novel high sensitivity cTnI assay [22], and longer range outcomes. The previous data have demonstrated that PAPP-A is a strong predictor of short-term (6 months) adverse events in patients with ACS [31,32]. Our data are similar and show the utility of an elevated PAPP-A as both an intermediate and long-term risk factor. These data also support the recent publication that PAPP-A is a predictor of long-term (median 9 years) all-cause mortality in patients with chronic stable angina pectoris [33], and thus provides indirect support for the notion that decreasing PAPP-A concentrations may be of therapeutic benefit. A recent study [34], demonstrated that high dose atorvastatin significantly decreased PAPP-A concentrations 1 month after an ACS event, and this observation raises the question of whether therapies targeting a reduction in PAPP-A might provide benefits over those targeting reduction in CRP levels after ACS [35,36]. Conversely, treatment with heparin during an acute event may increase plasma PAPP-A levels which may mask or alter the underlying pathophysiology [23,24]. Fortunately, during the time when this cohort was studied initially, heparin was not administered nearly as aggressively as it is today. However, we tried to avoid this confounding effect of heparin on PAPP-A concentrations by using the earliest specimen available. In the subgroup that did receive heparin therapy the peak PAPP-A concentration was significantly higher as compared to those that did not, lending support to these previous reports [23,24].

An intriguing finding was that PAPP-A measurement in those with acutely evolving myocardial injury (as characterized by the hs-cTnI concentration change) can identify those at increased probability for death. It also suggests that when a high sensitivity cTnI assay is employed to define elevations, it enriches the population being evaluated with patients potentially at risk. Once issues are addressed such as biological variability, and with it the optimal values to use to define significant changes, this sort of approach may be even more potent. Better pre hoc stratification may be very helpful for many biomarkers intended for risk stratification post events and might further amplify the importance of new high sensitivity troponin approaches. Conversely, it may make prognostication in those without cTn elevations far less common since a larger percentage will likely be without significant

coronary artery disease. It may be for that reason that PAPP-A was not prognostic in those without changing hs-cTnI values; however the small number of subjects with no change in hs-cTnI ($n=66$) in this cohort precludes a definitive statement in this regard.

There are several limitations to our study. First, the prevalence of statin use was not documented in our study population, yet this would be expected to be small considering that the utility rate was low back in 1996. Second, the utility of measuring PAPP-A in conjugation with NT-proBNP/BNP and other cardiovascular biomarkers needs to be addressed as measurement of the natriuretic peptides has a documented role in prognostication for patients presenting with ACS. Third, the hsPAPP-A assay utilized here measures uncomplexed PAPP-A and the PAPP-A/proMBP complex in equimolar concentration, yet it appears that only uncomplexed PAPP-A is released during ACS, thus future assays only measuring the uncomplexed version may provide additional benefit. Fourth, the clinical use of heparin may increase the PAPP-A concentrations and thus could mask the underlying pathophysiological release of PAPP-A. We have tried to reduce this effect by using the earliest specimen in our analysis, however, this issue needs to be addressed by properly conducted studies assessing this time-dependent release of PAPP-A by heparin therapy. Thus it is imperative that prospective studies are undertaken in ACS populations with specimens obtained prior to heparin administration, as well as measuring all available in use clinical laboratory cardiac biomarkers (i.e., CRP, NT-proBNP/BNP, cTn) in these studies before the role of PAPP-A can be conclusively determined. Finally, a reference interval has not been established for the hsPAPP-A assay nor do we have data on the long-term effect of storage on PAPP-A concentrations.

Future studies are required not only for the validation of PAPP-A in risk stratification but will also serve to optimize both the collection time and cut-off concentrations for PAPP-A in the management of patients with ACS.

Acknowledgments

This study was funded by CIHR. Reagents were provided as an unrestricted grant by Beckman Coulter. Special thanks to Dr. Ed Young for input on heparin therapy and the Clinical Research and Clinical Trials Laboratory, Hamilton for performing the biomarker measurements.

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Fig. 1.

Flow diagram for the selection of subjects for the health outcome analyses.

Fig. 2. Serial time profile of PAPP-A after onset of pain (1081 specimens: 0–3 h $n=228$; 4–5 h $n=227$; 6–9 h $n=262$; 10–23 h $n=180$; 24 h $n=184$) (A). Dot plot of presentation PAPP-A concentrations in each tertile (note 9 data points are >10 mIU/L in tertile 3 and are not included in this graph) (B).

Fig. 3.

Survival curves up to 2 years based on tertile analysis with baseline PAPP-A. (Tertile 1: PAPP-A<0.92; Tertile 2: PAPP-A 0.92–1.62; Tertile 3: PAPP-A>1.62 mIU/L) (A). Long-term survival, 10 years after presentation (B).

Fig. 4. Two-year survival curves in the group of subjects ($n=120$) with changing hs-cTnI concentrations, stratified by baseline PAPP-A tertiles.

Table 1

Study cohort characteristics.

Demographics	Total (n=320)
Males (%)	192 (60%)
Median age (IQR)	64 (51–74)
1996 AMI diagnosis	54 (16.9%)
# of PAPP-A measurements	
Median (IQR)	2 (1–5)
Earliest PAPP-A concentration (mIU/L)	
Median (IQR)	1.21 (0.80–1.97)
Time from pain onset for Early PAPP-A	
Median hours (IQR)	4 (2–9)
Earliest cTnI concentration (3g/L)	
Median (IQR)	0.01 (0.01–0.04)
Earliest hs-cTnI concentration (ng/L)	
Median (IQR)	10.8 (5.0–54.7)
Death within 2 years	50 (16%)
Death within 10 years	141 (44%)

Table 2

Spearman correlation of PAPP-A with other biomarkers in overall population and in non-heparin subgroup.

Biomarker	Overall population		No heparin (<i>n</i> =250)	
	PAPP-A <i>r</i>	<i>p</i> -value	PAPP-A <i>r</i>	<i>p</i> -value
Early cTnI	0.08	0.18	0.14	0.02
Early hs-cTnI	0.09	0.09	0.13	0.04

Table 3

Characteristics of group based on PAPP-A tertiles.

Variable	Tertile 1 (<i>n</i> =104) (<0.92 mIU/L) median (IQR): 0.70 (0.52–0.79)	Tertile 2 (<i>n</i> =108) (0.92–1.62 mIU/L) median (IQR): 1.19 (1.07–1.40)	Tertile 3 (<i>n</i> =108) (N1.62 mIU/L) median (IQR): 2.37 (1.96–3.87)	<i>p</i> -value
Sex				
Male	49	74	69	<0.01
Female	55	34	39	
History of MI/HF				
Yes	36	36	42	0.67
No	68	72	66	
ASA				
Yes	27	28	21	0.43
No	77	80	87	
Heparin treatment				
	23	21	26	0.68
	81	87	82	
1996 MI diagnosis				
Yes	12	17	25	0.07
No	92	91	83	
Time from onset for earliest PAPP-A measurement				
Median hours (IQR)	4 (2–9)	4 (2–8)	4 (2–8)	0.42
Earliest cTnI				
Median µg/L (IQR)	0.01 (0.00–0.03)	0.01 (0.00–0.04)	0.01 (0.00–0.06)	0.40
Peak cTnI				
Median µg/L (IQR)	0.02 (0.01–0.12)	0.02 (0.01–0.14)	0.03 (0.01–0.41)	0.24
Earliest hs-cTnI				
Median ng/L (IQR)	8.11 (4.86–41.4)	9.64 (4.34–38.3)	19.5 (6.04–87.1)	0.04

Table 4

Proportional hazard model of time to death 2 years after ED presentation.

Model	Tertile	Hazard ratio relative to tertile 1	95% CI	p-value
Crude	2	1.90	0.84–4.30	0.123
	3	2.96	1.38–6.35	0.005
Model 1	2	1.75	0.77–3.98	0.182
	3	2.23	1.04–4.80	0.040
Model 2	2	1.82	0.80–4.14	0.154
	3	2.15	1.00–4.63	0.050

Model 1: adjusted for age and sex.

Model 2: adjusted for age, sex, presentation cTnI 0.02 µg/L.